EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Scientific Opinion on the substantiation of a health claim related to beta-palmitate and increased calcium absorption pursuant to Article 14 of Regulation (EC) No 1924/2006

EFSA Publication

Link to article, DOI:
10.2903/j.efsa.2011.2289

Publication date:
2011

Document Version
Publisher's PDF, also known as Version of record

European Food Safety Authority

EFSA Journal 2011;9(7):2289

SCIENTIFIC OPINION

Scientific Opinion on the substantiation of a health claim related to beta-palmitate and increased calcium absorption pursuant to Article 14 of Regulation (EC) No 1924/2006[1]

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Following an application from IDACE, submitted pursuant to Article 14 of Regulation (EC) No 1924/2006 via the Competent Authority of France, the Panel on Dietetic Products, Nutrition and Allergies was asked to deliver an opinion on the scientific substantiation of a health claim related to beta-palmitate and increased calcium absorption. The scope of the application was proposed to fall under a health claim referring to children's development and health. The food constituent that is the subject of the health claim is beta-palmitate, a structured triglyceride with a high content of palmitic acid at the sn-2 (middle or beta) position of the glycerol backbone. Beta-palmitate is considered to be sufficiently characterised. The claimed effect is "beta palmitate enrichment contributes to increase calcium absorption". The target population proposed by the applicant is infants from birth to 12 months of age, including healthy infants consuming follow-on formula, preterm infants and infants needing foods for particular nutritional uses including foods for special medical purposes. The Panel considers that an increase in calcium absorption might be a beneficial physiological effect. In weighing the evidence, the Panel took into account the biological plausibility of the mechanism by which beta-palmitate could exert the claimed effect and that three small human intervention studies in preterm and term infants provided some evidence that a higher degree of palmitic acid in the sn-2 position of formula triglycerides may increase calcium absorption by decreasing faecal calcium excretion as calcium soaps, albeit a significant effect on calcium absorption was demonstrated in one study only. The Panel concludes that the evidence provided is insufficient to establish a cause and effect relationship between the consumption of beta-palmitate and an increase in calcium absorption.

KEY WORDS

Beta-palmitate, calcium absorption, saturated fatty acids, soaps, infants, infant formula, health claims.

[1] On request from the Competent Authority of France following an application by IDACE, Question No EFSA-Q-2008-172, adopted on 30 June 2011.

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[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on Claims: Carlo Agostoni, Jean-Louis Bresson, Susan Fairweather-Tait, Albert Flynn, Ines Golly, Marina Heinonen, Hannu Korhonen, Martinus Løvik, Ambroise Martin, Hildegard Przyrembel, Seppo Salminen, Yolanda Sanz, Sean (J.J.) Strain, Inge Tetens, Hendrik van Loveren and Hans Verhagen for the preparatory work on this scientific opinion.

Suggested citation: EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Scientific Opinion on the substantiation of a health claim related to beta-palmitate and increased calcium absorption pursuant to Article 14 of Regulation (EC) No 1924/2006. EFSA Journal 2011;9(7):2289. [16 pp.]. doi:10.2903/j.efsa.2011.2289. Available online: www.efsa.europa.eu/efsajournal

SUMMARY

Following an application from IDACE, submitted pursuant to Article 14 of Regulation (EC) No 1924/2006 via the Competent Authority of France, the Panel on Dietetic Products, Nutrition and Allergies was asked to deliver an opinion on the scientific substantiation of a health claim related to beta-palmitate and increased calcium absorption.

The scope of the application was proposed to fall under a health claim referring to children's development and health.

The food constituent that is the subject of the health claim is beta-palmitate, a structured triglyceride with a high content of palmitic acid at the sn-2 (middle or beta) position of the glycerol backbone. The Panel considers that beta-palmitate is sufficiently characterised.

The claimed effect is "beta palmitate enrichment contributes to increase calcium absorption". The target populations as proposed by the applicants is infants from birth to 12 months of age, including healthy infants consuming follow-on formula, preterm infants and infants who need other foods for particular nutritional uses, including foods for special medical purposes. The Panel considers that an increase in calcium absorption might be a beneficial physiological effect.

Higher relative calcium absorption from human milk is in part explained by the preferential positioning of palmitic acid at the sn-2 position of dietary triglycerides from which it is not released by pancreatic lipase but absorbed as sn-2-monoglyceride. In consequence less calcium soaps are formed with free long-chain saturated fatty acids in the intestine and less are excreted in the faeces.

The applicant provided 10 human and 6 animal studies for the scientific substantiation of the claim.

Of the 10 references in humans, four were unrelated to beta-palmitate and/or addressed health outcomes other than the claimed effect. One study investigated faecal calcium composition in healthy term infants, but did not assess calcium absorption. The Panel considers that no conclusions can be drawn from these references for the scientific substantiation of the claim. A systematic review of human intervention studies, comparing the effects of infant formulae with the addition of palmolein (i.e. palmitic acid positioned at sn-1 or sn-3) to formulae devoid of palmolein, included four controlled intervention studies conducted with beta-palmitate and which were also provided separately by the applicant. These studies were considered individually by the Panel.

The four studies were conducted on preterm or term infants. One study assessed the effects of beta-palmitate on bone mineral content (BMC) and density (BMD). The Panel notes that no adjustments were made for possible confounding factors and it is unclear whether a single measurement of BMC and BMD at the age of three months can be used as proxy for the determination of calcium absorption. The Panel considers that this study does not provide evidence that beta-palmitate increases calcium absorption and that no conclusions can be drawn from this reference for the scientific substantiation of the claim. The three remaining studies used calcium balance methods and included measures of calcium absorption: one short-term human intervention study in term newborn infants provided evidence that a high degree of palmitic acid in the sn-2 position of formula triglycerides may increase calcium absorption, whilst the two other studies, did not show such an effect, possibly because of insufficient sample sizes. However, these two studies showed that a high degree of palmitic acid in the sn-2 position of dietary triglycerides resulted in a significant decrease in faecal calcium excretion as calcium soaps.

The applicant also provided six animal studies, only two of which (in rats) dealt with the effects of beta-palmitate on calcium absorption. The Panel notes that results from studies in rats do not provide adequate information about the effects of beta-palmitate on calcium absorption or excretion in

humans and considers that no conclusions can be drawn from these studies for the scientific substantiation of the claim.

In weighing the evidence, the Panel took into account the biological plausibility of the mechanism by which beta-palmitate could exert the claimed effect and that three small human intervention studies in preterm and term infants provided some evidence that a higher degree of palmitic acid in the sn-2 position of formula triglycerides may increase calcium absorption by decreasing faecal calcium excretion as calcium soaps, albeit a significant effect on calcium absorption was demonstrated in one study only.

The Panel concludes that the evidence provided is insufficient to establish a cause and effect relationship between the consumption of beta-palmitate and an increase in calcium absorption.

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BACKGROUND

Regulation (EC) No 1924/2006[4] harmonises the provisions that relate to nutrition and health claims, and establishes rules governing the Community authorisation of health claims made on foods. As a rule, health claims are prohibited unless they comply with the general and specific requirements of this Regulation, are authorised in accordance with this Regulation and are included in the lists of authorised claims provided for in Articles 13 and 14 thereof. In particular, Articles 14 to 17 of this Regulation lay down provisions for the authorisation and subsequent inclusion of reduction of disease risk claims and claims referring to children's development and health in a Community list of permitted claims.

According to Article 15 of this Regulation, an application for authorisation shall be submitted by the applicant to the national competent authority of a Member State, which will make the application and any supplementary information supplied by the applicant available to the European Food Safety Authority (EFSA).

STEPS TAKEN BY EFSA:

- The application was received on 14/02/2008.
- The scope of the application was proposed to fall under a health claim referring to children's development and health.
- During the check for completeness[5] of the application, the applicant was requested to provide missing information on 26/03/2008, 07/05/2010, 25/02/2011.
- The applicant provided the missing information on 24/04/2008, 08/04/2010, 04/11/2010, 28/01/2011, 21/03/2011.
- The scientific evaluation procedure started on 30/03/2011.
- During the meeting on 30/06/2011, the NDA Panel, having evaluated the data submitted, adopted an opinion on the scientific substantiation of a health claim related to beta-palmitate and increased calcium absorption.

TERMS OF REFERENCE

EFSA is requested to evaluate the scientific data submitted by the applicant in accordance with Article 16 of Regulation (EC) No 1924/2006. On the basis of that evaluation, EFSA will issue an opinion on the scientific substantiation of a health claim related to: beta-palmitate and increased calcium absorption.

EFSA DISCLAIMER

The present opinion does not constitute, and cannot be construed as, an authorisation to the marketing of beta-palmitate, a positive assessment of its safety, nor a decision on whether beta-palmitate is, or is not, classified as a foodstuff. It should be noted that such an assessment is not foreseen in the framework of Regulation (EC) No 1924/2006.

[4] European Parliament and Council (2006). Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. Official Journal of the European Union OJ L 404, 30.12.2006. Corrigendum OJ L 12, 18.1.2007, p. 3–18.

[5] In accordance with EFSA "Scientific and Technical guidance for the Preparation and Presentation of the Application for Authorisation of a Health Claim"

It should also be highlighted that the scope, the proposed wording of the claim, and the conditions of use as proposed by the applicant may be subject to changes, pending the outcome of the authorisation procedure foreseen in Article 17 of Regulation (EC) No 1924/2006.

INFORMATION PROVIDED BY THE APPLICANT

Applicant's name and address: IDACE, Rue de l'Association, 50 1000 – Brussels, Belgium.

Food/constituent as stated by the applicant

According to the applicant, the food constituent of the health claim is beta-palmitate.

Health relationship as claimed by the applicant

According to the applicant, beta-palmitate contributes to an increased calcium absorption in infants receiving a formula enriched with this lipid source.

Wording of the health claim as proposed by the applicant

The applicant proposed the following wording for the health claim: "Beta palmitate enrichment contributes to increase calcium absorption" or alternatively "beta-palmitate contributes to/is involved in/participates to/increase absorption of calcium/increased calcium absorption."

Specific conditions of use as proposed by the applicant

According to the applicant, the target population are infants from birth up to 12 months, to the exception of well-being infants fed Infant Formulas as defined by Directive 91/321/EC and 2006/141/EC, for which authorised claims and conditions of use are already fixed by the annex IV of these directives. According to the applicant, formulas bearing this claim will contain a beta-palmitate concentration minimum 35 % of the total palmitic acid.

ASSESSMENT

1. Characterisation of the food/constituent

The food constituent that is the subject of the health claim is beta-palmitate.

Beta-palmitate is a structured triglyceride with a high content of triglycerides with palmitic acid at the sn-2 (middle or beta) position of the glycerol backbone. It is intended for use as an ingredient in formulae for infants and to be a part of the lipid fraction in these formulae by replacing other triglycerides within the range of lipids defined by the appropriate European Directives (follow-on formula) or by nutritional considerations (e.g., formula for preterm infants, food for special medical purposes (FSMP)).

Beta-palmitate is produced from unnamed vegetable oils by concentration, fractionation, blending and enzymatic esterification (45 to 80 % of total palmitic acid is esterified at the sn-2 position). The sn-1-3 positions are predominantly occupied by unsaturated fatty acids, primarily oleic acid (C18:1). The main triglycerides present in beta-palmitate are 1,2-dipalmitoyl 3-oleyl triglyceride (PPO) and 1,3-dioleyl 2-palmitoyl triglyceride (OPO).

According to the specifications from two manufacturers, beta-palmitate is a lightly yellow oil at 35 ºC and contains >98 % triglycerides; 28-60 % of total fatty acids are palmitic acid of which ≥50 % is in the sn-2 position, 30-60 % are oleic acid, <2 % are trans fatty acids and <0.1 % are free fatty acids. The peroxide value is ≤2.0 meq O_2/kg. The ratio of saturated fatty acids (SFAs) to mono-unsaturated

fatty acids (MUFAs) to poly-unsaturated fatty acids (PUFAs) measured in batches of beta-palmitate from the last five years was 40-50 : 45-50 : 5-7. Beta-palmitate is stabilised using antioxidant systems suitable for use in infant formula in the EU.

As stated by the applicant, beta-palmitate shall replace conventional triglycerides in formulae intended for infants to the extent that at least 35 % of the total palmitic acid content is in the sn-2 position, which means that the amount of beta-palmitate used in a food would depend on both the total fat content and its fatty acid composition.

The fatty acid content and the positioning of fatty acids on the glycerol backbone can be measured by established methods.

The Panel considers that the food constituent, beta-palmitate, which is the subject of the health claim, is sufficiently characterised.

2. Relevance of the claimed effect to human health

The claimed effect is "beta palmitate enrichment contributes to increase calcium absorption".

The target population proposed by the applicant is infants from birth to 12 months of age, including healthy infants consuming follow-on formula, preterm infants and infants who need other foods for particular nutritional uses (FPNUs) including foods for special medical purposes (FSMPs) because of diseases or medical conditions.

Inadequate dietary calcium intake, impaired calcium absorption and low calcium retention may contribute to impaired bone development in early life.

Improved absorption of a nutrient might be considered as a beneficial physiological effect where absorption is a limiting factor for the maintenance of an adequate status of the nutrient and where the absorbed nutrient can be utilised. The absorption of calcium can be a limiting factor in preterm infants in order to achieve the fetal accretion rate for calcium of 90-120 mg/kg/day (Atkinson and Tsang, 2005), in healthy term infants in order to achieve the retention of about 200 mg/day (Fomon and Nelson, 1993) and in infants with disturbances of lipid digestion which can result in insufficient calcium in the body to meet the demands of growing bone.

The Panel considers that an increase in calcium absorption might be a beneficial physiological effect.

3. Scientific substantiation of the claimed effect

Active calcium absorption occurs mostly in the duodenum and the proximal jejunum and is subject to physiological and nutritional regulation by the vitamin D endocrine system, whilst non-saturable calcium absorption occurs throughout the small intestine following a concentration gradient. Percentage calcium absorption is greater from human milk than from formula (term infants between 8-122 days of age: 58±17 % from human milk and 38±16 % from cow's milk based formulae; preterm infants: 73-79 % and 40-72 %, respectively) (Atkinson and Tsang, 2005; Droese and Stolley, 1967; Fomon and Nelson, 1993; Widdowson, 1965). Lower calcium absorption from formula is compensated for by higher calcium concentrations in formula leading to similar absolute calcium retention (Fomon and Nelson, 1993). Higher relative calcium absorption from human milk is in part explained by the preferential positioning of palmitic acid at the sn-2 position of dietary triglycerides from which it is not released by pancreatic lipase but absorbed as sn-2-monoglyceride (Innis, 1992; Innis et al., 1994; Martin et al., 1993; Tomarelli et al., 1968). In consequence, less calcium soaps are formed with free long-chain SFAs in the intestine and, because of their insolubility, are excreted in the faeces (Quinlan et al., 1995). Early studies confirmed that sn-2 positioning of palmitate enhanced fat digestibility and absorption in formulae (Filer et al., 1969; Tomarelli et al., 1968).

The applicant provided six animal studies (rat, piglet) on the effects of beta-palmitate on fat absorption and plasma lipids (de Fouw et al., 1994; Enzymotec Ltd, unpublished; Innis and Dyer, 1997; Innis et al., 1997; Lien et al., 1997; Sanders et al., 2001). Only two of these (in rats) dealt with calcium absorption (de Fouw et al., 1994) or faecal calcium excretion (Lien et al., 1997). The Panel notes that results from studies in rats do not provide adequate information about the effects of beta-palmitate on calcium absorption or excretion in humans and considers that no conclusions can be drawn from these studies for the scientific substantiation of the claim.

The applicant performed a literature search in Medline to identify publications which evaluated the effects of beta-palmitate-enriched formulas in infants. References which reported on the effects of beta-palmitate in adults were excluded.

Of the 10 references reporting on human data and which were identified by the applicant as being pertinent to the claim, four were unrelated to beta-palmitate (Innis et al., 1994; Quinlan et al., 1995) and/or addressed health outcomes (i.e., regurgitation, fat absorption, stool consistency, serum lipoprotein fatty acids) other than the claimed effect (i.e. calcium absorption) (Chevallier et al., 2009; Innis et al., 1994; Nelson and Innis, 1999; Quinlan et al., 1995). Lopez-Lopez et al. (2001) investigated faecal calcium, magnesium and fatty acid composition in healthy term infants, but did not assess calcium absorption. The Panel considers that no conclusions can be drawn from these references for the scientific substantiation of the claim.

A systematic review of human intervention studies on the effects of infant formulae with the addition of palmolein (i.e. palmitic acid positioned at sn-1 or sn-3) compared to formulae devoid of palmolein on intestinal fractional absorption of fat, palmitic acid and calcium, and on bone mineral content (BMC) and bone mineral density (BMD) was also provided (Koo et al., 2006). This systematic review included four controlled intervention studies conducted with beta-palmitate and which were also provided separately by the applicant. These studies are considered individually (Carnielli et al., 1995, 1996; Kennedy et al., 1999; Lucas et al., 1997).

The four studies were conducted on preterm (Carnielli et al., 1995; Lucas et al., 1997) or term (Carnielli et al., 1996; Kennedy et al., 1999) infants. Three of the studies (Carnielli et al., 1995, 1996; Lucas et al., 1997) used calcium balance methods and included measures of calcium absorption, whereas one study (Kennedy et al., 1999) assessed the effects of beta-palmitate on BMD and BMC.

Carnielli et al. (1995) conducted a randomised, double-blind, controlled, cross-over intervention study to address the effects of beta-palmitate on fat and mineral balance in 12 healthy preterm infants (birth weight 1.4±0.2 kg, weight during study period 2.1±0.3 kg, postnatal age 38±7 days). The intervention and control formulae only differed in the percentage of palmitic acid in the sn-2 position of dietary triglycerides (58 % in the intervention "beta" formula versus 9.8 % in the control formula without beta-palmitate) and were administered for one week each with no washout period. Balance studies (intake of formula and stool collection over three days, urine collection during one day) were undertaken during the last 72 h of each feeding period. Results are given as group mean±SE. Group mean values were compared by Student's t-test for paired data after analysis of variance showed no significant period effect for any studied variable. Correlations were assessed by simple linear regression. The Panel notes the small sample size of the study and that power calculations were not performed, so that this study may have been underpowered in relation to some of the outcomes tested.

Formula intake, faecal output, faecal fat and water content, intestinal transit time and urine production were not different between the two diet periods. During the "beta" formula feeding period, faecal calcium excretion was significantly lower (58.8±7.8 versus 82±9.9 mg/kg/day, $p<0.05$) and urine calcium excretion was significantly higher (4.0±0.7 versus 2.3±0.5 mg/kg/day, $p<0.05$) than during the period on the control formula. There were significant correlations between faecal calcium excretion and the faecal excretion of fat and fatty acids ($r=0.98$ for palmitic acid). Intestinal calcium

absorption as percentage of intake was not statistically different between the "beta" formula and the control (63.7±5.1 % versus 49.2±5.9 %).

The Panel notes that in this study, which had some methodological limitations (i.e., small sample size, no power calculations), a higher degree of palmitic acid in the sn-2 position of dietary triglycerides resulted in a statistically significant decrease of faecal calcium excretion, and that the small but statistically significant increase in urinary calcium excretion may indicate higher calcium absorption. However intestinal calcium absorption was not statistically significantly higher during the intake of the "beta" formula compared to the control formula.

Lucas et al. (1997) conducted a randomised, double-blind, controlled, parallel intervention study to investigate the effects of three different formulas on fat, palmitate and calcium absorption, and on the formation of soaps in the gut, in 30 preterm infants (birth weight <1500 g) who had started enteral feeding and were not breastfed. The three formulas were comparable for energy, macro- and micronutrient content, but differed with respect to the composition of the fat fraction. One formula contained beta-palmitate and 23.9 % of total fatty acids as palmitate, of which 73.9 % were in the sn-2 position, whilst the other two formulae did not contain beta-palmitate but rather 14.7 % palmitate, of which 8.4 % were in the sn-2 position (formula A) and 23.9 % palmitate, of which 27.8 % were in the sn-2 position (formula B), respectively. The Panel notes that the formula with beta-palmitate and the formula B contain the same percentage of fatty acids as palmitate but differ in the amount of palmitate in the sn-2 position, whereas in formula A both the percentage of fatty acids as palmitate and the percentage of palmitate in the sn-2 position differ with respect to the beta-palmitate formula. Thus formula A may not be an adequate control for the effect of changing the position of palmitate in the glycerol molecule of triglycerides in the beta-palmitate formula.

Infants were randomised before the age of 10 days to receive one of the three formulae (10 per group). Enteral feeds were increased according to individual feed tolerance. Conventional 3-5 day balance studies were performed in each infant after two weeks of complete enteral feeding (>150 mL of formula/kg/day), and a 24-h balance study using a dual calcium isotope technique was conducted during the first part of the balance study. The study groups were comparable for gestational age and birth weight. Randomisation was performed using assignments in sealed envelopes with a randomisation sequence based on permuted blocks of randomised length. The formulae were produced by one manufacturer and coded numerically. The codes were not known to the researcher and were not broken before the end of the study. Results were given as mean±SE. Between-group differences were tested by analysis of variance. When between-group differences were detected, comparisons between the beta-palmitate group and either group A or B were performed using Student's t-test. Study size was calculated to allow detection of 1.5 SD differences in the outcomes measured between the three groups after allowing for 20-30 % post-randomisation attrition of the sample. The Panel notes that power calculations are not sufficiently described (e.g., no information is provided regarding the primary outcome or α and β values used for power calculations) and that between-group comparisons were not corrected for multiple testing.

Seven infants in the beta-palmitate group, nine in the formula A group and six in the formula B group completed balance studies of at least three days, whereas seven infants in the beta-palmitate group, eight in the formula A group and seven in the formula B group completed the calcium isotope study and entered data analysis (completers only). Milk volume intake and intake of total fat and calcium were similar in the three groups at the start of the balance study and steady state gains in weight, length and head circumference were not different between the groups throughout the period on the assigned formulae. Infants consuming beta-palmitate formula excreted 3.3±0.7 % of their fat intake as fatty acid soaps and infants of the formula group B 7.2±0.8 ($p<0.01$) whilst the difference was not significant for the formula A group. Fractional absorption of calcium from the diet (relative recovery of the oral versus the intravenous calcium isotope in the 24 hour urine pool) was not statistically different between the beta-palmitate group (57.0±7.2 %) and the formula B group (40.0±4.5 %). The Panel notes that a statistically significant decrease in faecal excretion of fat intake (%) as fatty acid

soaps was observed in the beta-palmitate formula group compared to a formula (B) containing comparable amounts of palmitate but a lower percentage of palmitate in the sn-2 position. The Panel also notes that the study size may have been too small to detect differences between groups with respect to calcium absorption.

Carnielli et al. (1996) conducted a randomised, double-blind, controlled, parallel intervention study to investigate the effects of three different formulae (provided as the only feeding) on calcium absorption in 27 (nine per group) healthy, full-term male infants from birth until the age of five weeks. The three formulae were comparable with regard to energy, protein, total fat, calcium, phosphate and magnesium content, but differed in the percentage of palmitic acid in the sn-2 position of the dietary triglycerides: beta-palmitate formula "beta" with 23.9 mol% of total fatty acids as palmitic acid and 66 % of palmitic acid in the sn-2 position; beta-palmitate formula "intermediate" with 24 mol% of total fatty acids as palmitic acid and 39 % of palmitic acid in the sn-2 position; "regular" formula with 19.9 mol% of total fatty acids as palmitic acid and 13 % of palmitic acid in the sn-2 position. Randomisation was performed using assignments in sealed envelopes and investigators were blinded to the type of feeding. Three-day balance studies of infants were done at home at the age of about 4 weeks by a research nurse (72 h collection of faeces and 24 h collection of urine). Intestinal absorption was calculated by dividing the apparent amount absorbed by the intake. Data were given as group means±SD. Group means were compared by analysis of variance. Multiple post-hoc comparisons were analysed by the Tukey test. Correlations were calculated by simple linear regression. No power calculation was performed. All subjects randomised completed the balance studies.

Faecal fat excretion (both as percentage of faecal weight and in absolute values) was significantly lower in the "beta" group ($p<0.05$ for both comparisons) than in the "intermediate" and the "regular" groups. Faecal calcium excretion was significantly lower and intestinal calcium absorption was significantly higher in the "beta" group than in the two other groups ("intermediate" and "regular"): 43.3±18.1 versus 59.9±15.1 versus 68.4±22.3 mg/kg body weight per day ($p<0.05$), and 53.1±18.1 % versus 35.4±14.8 and 32.5±18.3 % ($p<0.05$), respectively. Calcium intake did not differ significantly between groups and calcium excretion in urine was not statistically significantly different between groups.

The Panel notes that a statistically significant decrease in faecal fat excretion and in faecal calcium excretion and a statistically significant increase in calcium absorption was observed in the beta-palmitate formula group compared to a beta-palmitate formula ("intermediate") containing comparable amounts of palmitate but a lower percentage of palmitate in the sn-2 position (66 versus 39 %), whilst there was no significant difference of calcium absorption between the "intermediate" beta-palmitate and the "regular" formula. These data suggest that the preferential positioning of palmitic acid at the sn-2 position of the glycerol molecule could have decreased faecal loss of calcium and increased calcium absorption possibly by enhancing intestinal absorption of palmitic acid.

Kennedy et al. (1999) performed a randomised, double-blind, controlled, parallel intervention study to investigate the effects of beta-palmitate on skeletal mineral deposition and stool consistency in 203 formula-fed healthy newborns. Subjects were enrolled within the first eight days of life and randomised to receive either a formula with beta-palmitate (n=100) or a control formula without beta-palmitate (n=103) for 12 weeks. One hundred and twenty breast-fed infants were also studied from 10 to 12 weeks of age. The beta-palmitate and control formulas were almost comparable in composition and percentage of fatty acids as palmitate (about 20 %), but differed in the percentage of palmitic acid in the sn-2 position (50 % in the beta-palmitate formula *vs.* 12 % in the control formula). The beta-palmitate formula also contained 4 % higher energy and 7 % higher fat content than the control formula. Sample size calculations (120 per group) were based on differences in stool hardness and constipation between the groups at 5 % significance and 80 % power. This sample size was also sufficient to detect a difference of 0.365 SD in BMC measured at the radius by single photon absorptiometry (SPA). A sample size of 40 per group was sufficient to detect a difference of 0.6 SD in

the measurement of BMC and BMD by dual-energy X-ray absorptiometry (DXA) at 5 % significance and 80 % power. Differences between groups were examined using Student's t-test for normally distributed data. Analyses were performed on an intention-to-treat basis, then separately for those infants who completed 12 weeks of the trial diet. Analysis of variance was used to examine differences in outcome variables between the three groups (two formulae, one breast-fed) with post-hoc pair-wise comparisons by Bonferroni tests. Primary outcomes were radius and whole BMC and BMD at the age of 12 weeks, and stool frequency, volume and consistency at the age of 6 and 12 weeks as reported by the mothers. The procedure for BMC and BMD determination was changed during the course of the study from SPA at the distal one-third site of the right ulna in the majority of study subjects, to whole-body DXA in 42, 40 and 69 infants of the beta-palmitate formula, control formula and breast-fed groups, respectively.

There were no statistically significant anthropometric differences between the two formula groups at randomisation and at 3, 6, and 12 weeks of age. Forty-three formula-fed infants stopped receiving the study formula before the end of week 12, 20 in the beta-palmitate group and 23 in the control-formula group. Of the 80 infants in both formula groups who completed 12 weeks of study formula feeding, 20 infants in the beta-palmitate group and 17 in the control formula group received complementary food at a median age of 10 weeks, leaving 60 infants in the beta-palmitate group and 63 infants in the control formula group who were exclusively fed with either study formula for 12 weeks.

There were no significant differences between the two formula groups in radial BMC measured by SPA, either before or after adjustment for body size and sex. Unadjusted whole-body BMC and BMD measured by DXA were not significantly different between the two formula groups, while after adjustment for sex and current body size (weight, length and bone area), there were significant differences in BMC and BMD ($p=0.05$ and $p=0.04$, respectively): beta-palmitate formula (n=42) BMC 128.1±9.7 g, BMD 0.244±0.019 g/cm^2; control formula (n=40) BMC 122.7±10.1 g, BMD 0.235±0.019 g/cm^2. When only infants with 12 completed weeks of consumption of study formula were compared, the differences became more apparent, $p=0.02$ and $p=0.009$ for BMC and BMD, respectively: the differences in absolute values between infants receiving study formulae for 12 weeks were for BMC 6.2 g (95 % CI 1.9, 10.5) and 0.012 g/cm^2 (95 % CI 0.004, 0.02) for BMD.

The Panel notes that the method of measuring bone mass changed during the course of the study. A statistically significant difference in both BMC and BMD between the groups assigned to different study formulae was found by the preferable DXA method in a sample size just sufficient to detect differences of 0.6 SD. The Panel notes, however, that the sample was not homogenous in that all infants had received the study formula for 12 weeks (34 of 42 in the beta-palmitate group and 37 of 40 in the control formula group). The Panel notes that no adjustments were made for possible confounding factors like stature of parents, dietary habits, sun exposure and calcium intake of mothers, smoking habits of parents and which may have had an impact on the whole-body BMC of an infant at birth and, therefore, during the first months of life. The Panel also notes that it has not been proven that a single measurement of BMC and BMD at the age of three months can be used as proxy for the determination of calcium absorption and that a higher value of BMC and BMD at that age in a sample of the beta-palmitate group can be attributed to better calcium absorption. The Panel considers that this study does not provide evidence that beta-palmitate increases calcium absorption and that no conclusions can be drawn from this reference for the scientific substantiation of the claim.

The Panel notes that no data are available in older infants and in infants with disturbances of lipid digestion.

The Panel notes that one short-term human intervention study in term newborn infants (Carnielli et al., 1996) provides evidence that a high degree of palmitic acid in the sn-2 position of formula triglycerides may increase calcium absorption, whilst two other studies do not show such an effect, possibly because of insufficient sample sizes (Carnielli et al., 1995; Lucas et al., 1997). However, these two studies showed that a high degree of palmitic acid in the sn-2 position of dietary

triglycerides resulted in a significant decrease in faecal calcium excretion as calcium soaps (Carnielli et al., 1995; Lucas et al., 1997).

In weighing the evidence, the Panel took into account the biological plausibility of the mechanism by which beta-palmitate could exert the claimed effect and that three small human intervention studies in preterm and term infants provided some evidence that a higher degree of palmitic acid in the sn-2 position of formula triglycerides may increase calcium absorption by decreasing faecal calcium excretion as calcium soaps, albeit a significant effect on calcium absorption was demonstrated in one study only.

The Panel concludes that the evidence provided is insufficient to establish a cause and effect relationship between the consumption of beta-palmitate and an increase in calcium absorption.

CONCLUSIONS

On the basis of the data presented, the Panel concludes that:

- The food constituent, beta-palmitate, which is the subject of the health claim, is sufficiently characterised.
- The claimed effect is "beta palmitate enrichment contributes to increase calcium absorption". The target population as proposed by the applicant is infants from birth to 12 months of age. An increase in calcium absorption might be a beneficial physiological effect.
- The evidence provided is insufficient to establish a cause and effect relationship between the consumption of beta-palmitate and an increase in calcium absorption.

DOCUMENTATION PROVIDED TO EFSA

Health claim application on beta-palmitate and increased calcium absorption pursuant to Article 14 of Regulation (EC) No 1924/2006 (Claim serial No: 0092_FR). March 2011. Submitted by IDACE.

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GLOSSARY/ABBREVIATIONS

BMC	Bone mineral content
BMD	Bone mineral density
CI	Confidence interval
DXA	Dual-energy X-ray absorptiometry
FPNU	Food for particular nutritional uses
FSMP	Food for special medical purposes
MUFA	Monounsaturated fatty acid
OPO	1,3-dioleoyl 2-palmitoyl triglyceride
PPO	1,2-dipalmitoyl 3-oleyl triglyceride
PUFA	Polyunsaturated fatty acid
SD	Standard deviation
SE	Standard error
SFA	Saturated fatty acid
SPA	Single photon absorptiometry